

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have also been amended to delete multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachment: Version With Markings Showing Changes Made

(Rev. 01/22/01)

VERSION WITH MARKINGS SHOWING CHANGES MADE

The specification has been amended to provide cross-referencing to the International Application.

The claims have been amended as follows:

3. (Amended) A method according to claim 1 [or 2], wherein the influence comprises contact between the mechanically intact or permeabilised living cells and a chemical substance and/or incubation of the mechanically intact or permeabilised living cells with a chemical substance.

4. (Amended) A method according to [any of claims 1-3]claim 1, wherein the cells comprise a group of cells contained within a spatial limitation.

5. (Amended) A method according to [any of claims 1-4]claim 1, wherein the cells comprises multiple groups of cells contained within multiple spatial limitations.

6. (Amended) A method according to [any of claims 1-5]claim 1, wherein the spatial limitations are spatial limitations arranged in one or more arrays on a common carrier.

8. (Amended) A method according to [any of claims 1-7]claim 1, wherein the redistribution results in quenching of fluorescence, the quenching being measure as a decrease in the intensity of the fluorescence.

9. (Amended) A method according to [any of claims 1-8]claim 1, wherein the redistribution results in energy transfer, the energy transfer being measure as a change in the intensity of the luminescence.

10. (Amended) A method according to [any of claims 1-8]claim 1, wherein the intensity of the light being recorded is a function of the fluorescence lifetime, polarization, wavelength shift, or other property which is modulated as a result of the underlying cellular response.

11. (Amended) A method according to [any of claims 1-10]claim 1, wherein the light to be measured passes through a filter which selects the desired component of the light to be measured and rejects other components.

12. (Amended) A method according to [any of claims 1-11]claim 1, wherein the fluorescence comes from a fluorophore encoded by and expressed from a nucleotide sequence harboured in the cells.

13. (Amended) A method according to [any of the preceding claims]claim 1, wherein the fluorescence comes from a luminescent polypeptide, such as GFP.

14. (Amended) A method according to [any of the preceding claims]claim 1, wherein the luminescent polypeptide could be a GFP selected from the group consisting of green fluorescent proteins having the F64L such as F64L-GFP, F64L-Y66H-GFp, F64L-S65T-GFP, and EGFP.

15. (Amended) A method according to [any of claims 1-14]claim 1, wherein the cells are selected from the group consisting of fungal cells, such as yeast cells; invertebrate cells including insect cells; and vertebrate cells, such as mammalian cells.

17. (Amended) A method according to [any of claims 1-16]claim 1, used as a screening program.

20. (Amended) A set of data relating to an influence on a cellular response in mechanically intact or permeabilised living cells, obtained by a method according to [any of claims 1-19]claim 1.

VERSION WITH MARKING TO SHOW CHANGES MADE

The paragraph beginning on page 40, line 12 has been amended as follows:

To construct the PKAc-F64L-S65T-GFP fusion, convenient restriction endonuclease sites were introduced into the cDNAs encoding murine PKAc (Gen Bank Accession number: M12303) and F64L-S65T-GFP (sequence disclosed in WO 97/11094) by polymerase chain reaction (PCR).

The PCR reactions were performed according to standard protocols with the following primers:

5'PKAc:

TTggACACAAgCTTTggACACCCTCAggATATgggCAACgCCgCCgCCgCCAAg (SEQ ID NO:19),

3'PKAc:

gTCATCTTCTCgAgTCTTTCAGgCgCgCCCAAACCTCAgTAAACTCCTTgCCACAC (SEQ ID NO:20)

5'GFP:

TTggACACAAgCTTTggACACggCgCgCCATgAgTAAaggAgAAgAACTTTTC (SEQ ID NO:21)

3'GFP:

gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT (SEQ ID NO:22).

The paragraph beginning on page 44, line 32 has been amended as follows:

EXAMPLE 2 Probe for detection of PKC activity

Construction of PKC-GFP fusion:

The probe was constructed by ligating two restriction enzyme treated polymerase chain reaction (PCR) amplification products of the cDNA for murine PKC α (GenBank Accession number:

M25811) and F64L-S65T-GFP (sequence disclosed in WO 97/11094) respectively. Taq®

polymerase and the following oligonucleotide primers were used for PCR;

5'mPKCα: TTggACACAAgCTTTggACACCCTCAggATATggCTgACgTTTACCCggCCAACg
(SEQ ID NO:23)

3'mPKCα:

gTCATCTTCTCgAgTCTTTCaggCgCgCCCTACTgCACTTTgCAAgATTgggTgC (SEQ ID
NO:24),

5'F64L-S65T-GFP:

TTggACACAAgCTTTggACACggCgCgCCATgAgTAAaggAgAAgAACTTTTC (SEQ ID
NO:25),

3'F64L-S65T-GFP:

gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT (SEQ ID
NO:26).

The paragraph beginning on page 47, line 13 has been amended as follows:

The human Erk1 gene (GenBank Accession number: X60188) was amplified using PCR
according to standard protocols with primers

Erk1-top

5'-TAGAATTCAACCATGGCGGCGGCGGGCG (SEQ ID NO:27)-3'

and Erk1-bottom/+stop

5'-TAGGATCCCTAGGGGGCCTCCAGCACTCC (SEQ ID NO:28)-3'.

The PCR product was digested with restriction enzymes EcoR1 and BamH1, and ligated into
pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with EcoR1 and

BamH1. This produces an EGFP-Erk1 fusion (SEQ ID NOs: 5 and 6) under the control of a CMV promoter.

The paragraph beginning on page 49, line 16 has been amended as follows:

Smad 2, a signal transducer, is a component of a signalling pathway that is induced by some members of the TGFbeta family of cytokines.

a) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC (SEQ ID NO:29)-3'

and Smad2-bottom/+stop

5'-GTGGTACCTTATGACATGCTTGAGCAACGCAC (SEQ ID NO:30)-3'.

The PCR product was digested with restriction enzymes EcoR1 and Acc65I, and ligated into pEGFP-C1 (Clontech; Palo Alto; GenBank Accession number U55763) digested with EcoR1 and Acc65I. This produces an EGFP-Smad2 fusion (SEQ ID NOs: 7 and 8) under the control of a CMV promoter.

b) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC (SEQ ID NO:31)-3'

and Smad2-bottom/-stop

5'-GTGGTACCCATGACATGCTTGAGCAACGCAC (SEQ ID NO:32)-3'.

The paragraph beginning on page 48, line 14 has been amended as follows:

EXAMPLE 5 Probes for detection of VASP redistribution.

Useful for monitoring signalling pathways involving rearrangement of cytoskeletal elements, e.g. to identify compounds which modulate the activity of the pathway in living cells. VASP, a phosphoprotein, is a component of cytoskeletal structures, which redistributes in response to signals that affect focal adhesions.

The human VASP gene (GenBank Accession number: Z46389) was amplified using PCR according to standard protocols with primers

VASP-top

5'-GGGAAGCTTCCATGAGCGAGACGGTCATC (SEQ ID NO:33)-3'

and VASP-bottom/+stop

5'-CCCGGATCCTCAGGGAGAACCCCGCTTC (SEQ ID NO:34)-3'.

The paragraph beginning on page 50, line 4 has been amended as follows:

EXAMPLE 7 Probes for detection of NFkappaB redistribution.

Useful for monitoring signalling pathways leading to activation of NFkappaB, e.g. to identify compounds which modulate the activity of the pathway in living cells.

NFkappaB, an activator of transcription, is a component of signalling pathways that are responsive to a variety of inducers including cytokines, lymphokines, and some immunosuppressive agents.

a) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers

NFkappaB-top

5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC (SEQ ID NO:35)-3'

and NFkappaB-bottom/+stop

5'-GTGGATCCTTAGGAGCTGATCTGACTCAGCAG (SEQ ID NO:36)-3'.

The PCR product is digested with restriction enzymes XhoI and BamHI, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with XhoI and BamHI. This produces an EGFP-NFkappaB fusion (SEQ ID NOs:13 and 14) under the control of a CMV promoter.

b) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers

NFkappaB-top

5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC (SEQ ID NO:37)-3'

and NFkappaB-bottom/-stop

5'-GTGGATCCAAGGAGCTGATCTGACTCAGCAG (SEQ ID NO:38)-3'.

The PCR product is digested with restriction enzymes XhoI and BamHI, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession number U55762) digested with XhoI and BamHI. This produces an NFkappaB-EGFP fusion (SEQ ID NOs: 15 and 16) under the control of a CMV promoter.

The paragraph beginning on page 54, line 1 has been amended as follows:

EXAMPLE 11 Probes for detection of PKC β 1 redistribution.

Useful for monitoring signalling pathways involving Protein Kinase C, e.g. to identify compounds which modulate the activity of the pathway in living cells.

PKC β 1, a serine/threonine protein kinase, is closely related to PKC α and PKC β 2 but not identical; it is a component of a signalling pathway which is activated by elevation of intracellular calcium concomitant with an increase in diacylglycerol species.

a) The human PKC β 1 gene (GenBank Accession number: X06318) was amplified using PCR according to standard protocols with primers

PKC β 1-top

GTCTCGAGGCAAGATGGCTGACCC (SEQ ID NO:39)

and PKC β 1-bottom

GTGGATCCCTACACATTAATGACAACTCTGGG (SEQ ID NO:40).